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# Tuning of membrane electrostatic properties by single chain sphingolipids sphingosine and sphingosine-1-phosphate: The effect on bilayer dipole potential

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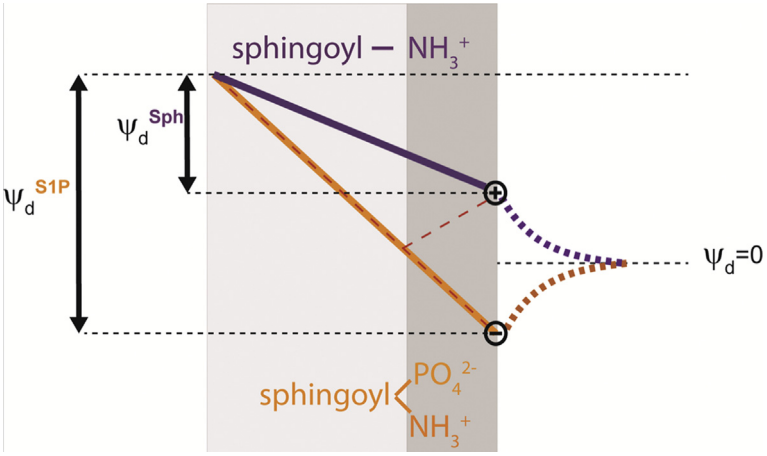
## HIGHLIGHTS

- Sphingosine and sphingosine-1 phosphate affect the membrane dipole potential.
- Mixtures of the two sphingolipids monotonously modulate membrane dipole potential.
- Dipole potential effects may be involved in cell fate regulation by sphingolipids.

## GRAPHICAL ABSTRACT

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## ABSTRACT

An important question in membrane biological chemistry is whether bioactive signaling lipids act only as second messenger ligands or also through an effect on bilayer physical properties. Sphingosine (Sph) and sphingosine-1-phosphate (S1P) are single-chained charged sphingolipids that have antagonistic functions in the "sphingolipid rheostat" which determines cell fate. Sph and S1P respectively promote apoptosis and cell growth. In the present study, the effects of these bioactive lipids on the dipole potential of the lipid bilayer were evaluated. We have investigated the effect of both sphingolipids, incorporated separately or together, in large egg phosphadidylcholine (EPC) unilamellar vesicles on the fluorescence of di-8-ANEPPS, a probe of dipole potential. Both sphingolipids promote a decrease of the dipole potential which is more pronounced for Sph than for S1P. This can be explained by the polarization properties of both the single-chain sphingoid backbone and its surrounding water molecules, as well as by the opposite net charges of the two sphingolipids. When both sphingolipids are present together, as occurs physiologically, the dipole potential varies monotonously over a significant range as a function of the Sph/S1P ratio. This suggests that both sphingolipids are able to tune the dipole potential of cell membranes as a function of their ratio, thereby possibly influencing the binding of surface proteins and the

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activity of transmembrane proteins. This furthers the idea that the two sphingolipids might exert their biological activity not only as second messenger ligands but also through their effect on lipid membrane physicochemical properties.



## Introduction

Lipids, apart from being responsible of the barrier properties of cell membranes, also have more specific functional roles. In particular, minor lipid components, often termed "bioactive lipids", act as second messengers [1]. Such lipids are mostly known to function as membrane-anchoring and/or activity-modulating ligands for specific receptors, adaptor proteins or signaling enzymes. However, it has been proposed several times that these lipids act in parallel through their effect on local properties of the membrane lipid bilayer, such as curvature, surface charge, local pH, fluidity and domain organization [2–5].

The two minor single-chain charged sphingolipids Sph and S1P (Fig. 1) are known to control cell fate, together with ceramide, through an ensemble of antagonistic mechanisms known as the "sphingolipid rheostat" [6–11]. The term "sphingolipid rheostat" was coined from the observation that the relative proportions of these sphingolipids rather than their absolute concentrations govern cell fate. Ceramide and Sph work as pro-apoptotic and anti-mitogenic signals while S1P work as survival, anti-apoptotic and mitogenic factor. The syntheses of all three lipids are metabolically interconnected as such: ceramide ↔ Sph ↔ S1P. The interconversion between Sph and S1P involves specific kinases and phosphatases tightly regulated by stress or growth effectors. The antagonistic biological activities of both sphingolipids have been correlated with their direct targeting of enzymes, cofactors or receptors involved in apoptotic and proliferation pathways [7,8]. However the possibility exists that the respective functions of both single chain phospholipids are also related to their direct effect on physicochemical properties of the lipid bilayer. Investigating the effect of such lipids on artificial membranes of defined composition is an efficient approach for evaluating this hypothesis.

A few studies have addressed the behavior of Sph and S1P in lipid bilayers [12]. As emphasized in our recent work, the effects of both sphingolipids appear to be antagonistic [13]. While Sph significantly increases the order of lipid bilayers [12–15], S1P either decreases [16] or only moderately increases [13] such order depending on lipid composition. Sph appears to promote the formation of gel phase microdomains [13,15] and to stabilize liquid-ordered domain [15] whereas S1P disrupts ordered domains [13]. The two single chain sphingolipid have opposite charges at physiological pH and thereby yield opposite effects on bilayer surface charges as measured through zeta potential [13].

Another physiologically relevant physicochemical property of lipid bilayers is the dipole potential [17–19]. The dipole potential is the electrical potential that occurs transversally between the water–lipid interface and the hydrocarbon interior. It is contributed by all polarized and polarizable chemical groups of lipids as well as by water next to and within the bilayer. For PC bilayer, it is positive inside the hydrophobic region due to the dominant contribution of oriented lipid-bound water molecules. Since the dipole potential has values of several hundreds of millivolts and occurs within distances of 1–2 nm [18,19], it corresponds to very important electrical field strength. Such field strength can influence the conformation and activity of intrinsic proteins [20] as well as the membrane binding of surface peptides and proteins [21]. In the present study we have investigated the effect of Sph and S1P, incorporated separately or together, on the dipole potential of EPC bilayers.

## Materials and methods

### Lipids

Egg yolk L-α-phosphatidylcholine (EPC), D-*erythro*-Sphingosine (Sph) and D-*erythro*-sphingosine-1-phosphate (S1P) were from Avanti Polar Lipids (Alabaster, AL). Di-8-ANEPPS and Texas Red DPPE (TR-PE) were from Invitrogen, Carlsbad, CA. S1P was first solubilized in ethanol (96%), vortexed, sonicated for 5 min and diluted 20 times in methanol to obtain a clear solution at 0.25 mg/mL. Other lipid stock solutions were in chloroform.

### Large unilamellar vesicles preparation

LUVs were prepared using the extrusion method as described [22], using HEPES 5 mM, NaCl 1 mM, pH 7.4 buffer. Lipids in chloroform, chloroform/methanol or ethanol/methanol were initially mixed to obtain the desired compositions. For fluorescence spectroscopy, di-8-ANEPPS was mixed with the lipids in the initial organic solution at a probe:lipid mole percent of 0.25%.

### Spectroscopic fluorescence measurements

Steady-state fluorescence measurements were carried out with a Cary Eclipse spectrofluorimeter (Varian Instruments, CA) equipped with a thermostated (±0.1 °C) cuvette holder. Quartz cuvettes were used. Excitation and emission slits were adjusted to 5 nm. All fluorescence measurements were carried out at a total lipid concentration of 0.2 mM. Samples were equilibrated for 5 min at the desired temperature.

## Results

### Separate effects of Sph and S1P on the dipole potential of EPC bilayers

We first studied the influence of Sph and S1P on the dipole potential of EPC. For this purpose we made use of LUVs incorporating the fluorescence probe di-8-ANEPPS incorporated into LUVs. It has been shown that the fluorescence intensity ratio $R_{ex} = I_{670/exc420}/I_{670/exc520}$ provides a direct measure of the dipole potential, independently of fluidity effects [23]. For lipid bilayers in the fluid phase, $R_{ex}$ is proportional to the dipole potential (in mV) [20,24]:

$$\Psi_d = \frac{R_{ex} + 0.3}{0.0043}$$

The $R_{ex}$ values for EPC LUVs in the presence of two concentrations of Sph and S1P and at various temperatures are shown in Table 1. Some of the samples described below (marked in Table 1) contain a limited fraction of gel phase [13]. The $R_{ex}$ value for EPC alone corresponds to a dipole potential value of ~367 mV, and is in the range usually found for fluid phase phosphatidylcholine [20,25]. Sph appears to significantly decrease $R_{ex}$, and therefore the dipole potential, in a concentration-dependent fashion. For S1P, the variation remains within the error bar. However all $R_{ex}$ values with S1P fall below the corresponding value without S1P. It is therefore